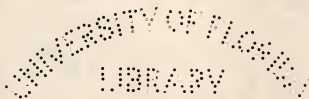


BIOLOGICAL ASSAY OF GELSEMIUM

By

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY



UNIVERSITY OF FLORIDA

August, 1938

ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Dr. B.V. Christensen, under whose supervision this work was done, for his valuable suggestions and thoughtful criticisms.

He also wishes to express his appreciation to the Graduate Council of the University of Florida for the Scholarships which aided materially in making this work possible.

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INTRODUCTION AND REVIEW OF THE LITERATURE

The need for a suitable assay for preparations of Gelsemium has been pointed out in a previous work (1) and a method was suggested which seemed to give satisfactory results. This paper gives data to further substantiate the belief that the pigeon emesis method, as previously outlined, can be used as a satisfactory measure of the activity of this drug.

Several important questions relating to the feasibility of the method have been studied and the data are presented here. It was felt that a restandardization of some of the preparations used in the previous paper and the standardization of a few new preparations would give interesting data. By restandardizing those preparations which had already been standardized an idea of the accuracy of the method could be obtained and the question of sensitiveness of pigeons to repeated injections could be studied. In order to have material for comparisons other methods of biological assay were tried and the results have been given here. Considerable study was devoted to the question as to what principle or principles contained in the drug were responsible for the emetic action. In order to settle this it was necessary to isolate the alkaloids as reported in the literature and to give doses of each of these sufficiently large to allow conclusions to be drawn. Although gelsemic acid (scopolletin) has been reported as inactive, injections of this were made so as to leave no doubt of its inability to cause emesis. It seemed that the knowledge of the cause of the emetic action would aid in judging the plausibility of the method.

The literature was thoroughly reviewed in the previous paper and a complete bibliography of all work reported up to that time was given. The review gave a discussion of the chemical investigations, the suggested chemical methods of assay as well as the suggested methods for biological assay.

Several articles of interest have appeared since the compilation of this review. Within the past few months Ramond-Hamet (2, 3, 4) has given some data concerning the action of the three crystalline alkaloids which have been reported. He states that sempervirine is related to sparteine in two effects, that sempervirine, gelsemine and gelsamine, all three, have the power to augment the hypertensive effects of adrenaline, that although gelsamine is the most toxic it has the least effect upon arterial pressure, and that gelsemine which has been considered as inactive for mammals by most workers, is the least toxic for guinea pigs, but that it does possess physiologic activity for this animal. Moisset (5) reports that in the dog no modification in the electrocardiogram follows small doses of fluidextract of Gelsemium while large doses hinder cardiac conductivity.

THE PIGEON EMESIS ASSAYS

EXPERIMENTAL PROCEDURE AND DATA

The procedure for this method was described in the previous report (1). The only change that has been made is in the suggested time of observation. A thirty minute period of observation was recommended at first, but after further study it was concluded that this period is longer than necessary and that twenty minutes is time enough to allow before calling a reaction negative since emesis is generally provoked in ten minutes or less. There were a few instances in which emesis occurred after twenty minutes but this was not characteristic, and in no case was the number sufficient to affect the final result. By shortening the observation period to twenty minutes the time of assay is decreased one third thus enabling the saving of considerable time over the entire period of assay.

The Minimum Emetic Dose (M.E.M.D.) is defined as that quantity, expressed in cc. per Kg. of total weight of pigeon, which, when injected intravenously, will cause emesis in seventy-five per cent of the pigeons injected, within 20 minutes.

Eight tinctures were used in this study. Five of these were used in the previous investigation and three were used only in this one. The sources of the five preparations were described in the previous paper but for the sake of completeness these are given here in tabular form together with the information about the remaining three preparations. Preparations 110, 114 and 116 are the ones which had not been standardized prior to this study.

TABLE 1

Tabulation of the Data Concerning Gelsemium Preparations used in Experimental Tests.

Preparation	Menstruum	Source
102	Approx. 60% alcohol.	From fresh cultivated drug.
104	N.F.VI	From a mixture of 5 samples crude drug obtained on the market.
106	N.F.VI	Same drug as for 102 after careful drying.
107	N.F.V	Manufacturer.
108	N.F.V	Manufacturer.
110	N.F.VI	From fresh Florida grown drug.
114	N.F.VI	From representative sample of 100 pound lot obtained on market.
116	N.F.VI	Same drug as for 110 after careful drying.

The pigeons used consisted of a lot of 85 ranging in weight from 250 to 400 Gm. which had not been used for injections of Gelsemium prior to this investigation. They were fed a diet prepared especially for pigeons by commercial feed producers with some green material at intervals and were allowed access to gravel. They were housed in a cage on the ground with ample protection against the weather. The health of the birds remained excellent throughout the entire experiment.

The M.Ea.D. of each preparation was determined following the procedure already described. Following this a redetermination of the M.Ea.D.

for three of the preparations was made and a third determination was made for one. The results of these standardizations are presented in the following tables, 2 to 18 inclusive. A summary table for each preparation showing the results previously obtained is included so that comparisons may be made more easily. The "b" tables give a summary of the results shown in the "a" tables. Table 19 gives a summary of tables 2 to 18.



TABLE 2

Preparation 102. The Average Minimum Emetic Dose as Previously Determined (1).

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90	4	4	
0.80	4	3	1
0.70	4	4	
0.60	4	3	1
0.55	8	7	1
0.50*	16	12	4
0.45	8	4	4
0.40	8	2	6
0.30	4	1	3

* Minimum Emetic Dose

TABLE 3 a

Preparation 102. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
280	0.70	Emesis	6
360	0.70	Emesis	17
305	0.70	No Emesis	20
265	0.70	Emesis	6
315	0.70	Emesis	8
290	0.70	Emesis	4
330	0.70	Emesis	8
285	0.70	Emesis	6
285	0.65	Emesis	10
355	0.65	Emesis	16
280	0.65	Emesis	11
345	0.65	Emesis	5
345	0.60	Emesis	6
330	0.60	Emesis	6
330	0.60	Emesis	10
360	0.60	Emesis	4
280	0.60	Emesis	4
315	0.60	No Emesis	20
300	0.60	Emesis	11
345	0.60	Emesis	15
295	0.55	Emesis	9
360	0.55	Emesis	4
375	0.55	Emesis	8
355	0.55	Emesis	7
270	0.55	Emesis	10
355	0.55	No Emesis	20
330	0.55	Emesis	12
315	0.55	Emesis	6
300	0.55	Emesis	5
340	0.55	Emesis	9
340	0.55	Emesis	11
310	0.55	Emesis	10
335	0.55	Emesis	9
360	0.55	Emesis	5
375	0.55	Emesis	5
370	0.55	Emesis	9
325	0.50	No Emesis	20
350	0.50	Emesis	8

(Continued next page)

TABLE 3 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
345	0.50	Emesis	8
360	0.50	Emesis	4
330	0.50	Emesis	4
290	0.50	Emesis	10
325	0.50	Emesis	15
320	0.50	Emesis	3
255	0.50	Emesis	11
300	0.50	No Emesis	20
280	0.50	No Emesis	20
350	0.50	Emesis	10
385	0.50	No Emesis	20
335	0.50	No Emesis	20
365	0.50	Emesis	4
350	0.50	Emesis	8
320	0.50	Emesis	8
265	0.50	No Emesis	20
310	0.50	No Emesis	20
285	0.50	No Emesis	20
340	0.50	Emesis	16
315	0.50	No Emesis	20
355	0.50	No Emesis	20
345	0.50	Emesis	5
375	0.50	Emesis	2
370	0.50	Emesis	15
325	0.50	Emesis	3
370	0.50	Emesis	8
380	0.50	Emesis	4
305	0.50	Emesis	11
325	0.50	No Emesis	20
340	0.50	Emesis	5
350	0.45	Emesis	12
340	0.45	Emesis	13
300	0.45	No Emesis	20
365	0.45	Emesis	8
380	0.45	Emesis	5
320	0.45	Emesis	8
320	0.45	Emesis	8
300	0.45	No Emesis	20
320	0.45	Emesis	9
330	0.45	Emesis	7
280	0.45	No Emesis	20

(Continued next page)

TABLE 3 a (Continued)

Weight of Pigeon in Grams	Dose cc./kg.	Results	Time, in Minutes, Observed
285	0.45	Emesis	5
300	0.45	No Emesis	20
335	0.45	Emesis	17
290	0.45	Emesis	6
330	0.45	Emesis	12
290	0.45	Emesis	5
310	0.45	Emesis	8
330	0.45	Emesis	20
325	0.45	Emesis	6
290	0.45	No Emesis	20
315	0.45	No Emesis	20
305	0.45	Emesis	7
315	0.45	Emesis	10
330	0.45	No Emesis	20
300	0.45	Emesis	5
340	0.45	Emesis	12
375	0.45	Emesis	10
335	0.45	Emesis	10
335	0.45	Emesis	20
300	0.45	Emesis	9
360	0.45	Emesis	9
315	0.40	Emesis	20
290	0.40	No Emesis	20
350	0.40	Emesis	12
310	0.40	No Emesis	20
295	0.40	No Emesis	20
375	0.40	Emesis	2
345	0.40	No Emesis	20
290	0.40	No Emesis	20
325	0.40	No Emesis	20
360	0.40	No Emesis	20
340	0.40	No Emesis	20
340	0.40	No Emesis	20
335	0.40	No Emesis	20
325	0.40	No Emesis	20
380	0.40	Emesis	6
320	0.40	Emesis	4
330	0.40	Emesis	10
300	0.40	No Emesis	20
310	0.40	Emesis	9
340	0.40	No Emesis	20

TABLE 3 b

Summary of Table 3 a. The Average Minimum Emetic Dose of Preparation 102.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.70	8	7	1
0.65	4	4	
0.60	8	7	1
0.55	16	15	1
0.50	32	21	11
0.45*	32	25	7
0.40	20	7	13

* Minimum Emetic Dose

TABLE 4

Preparation 104. The Average Minimum Emetic Dose as Previously Determined (1).

Dose cc./kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.95	8	8	
0.90*	16	13	3
0.85	12	7	5
0.80	12	6	6
0.70	8	3	5
0.60	4		4

* Minimum Emetic Dose

TABLE 5 a

Preparation 104. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose	Results	Time, in Minutes, Observed
310	0.90	Emesis	9
275	0.90	Emesis	5
295	0.90	Emesis	9
345	0.90	Emesis	9
300	0.90	Emesis	12
265	0.90	Emesis	10
280	0.90	Emesis	5
325	0.90	No Emesis	20
250	0.90	Emesis	9
270	0.90	No Emesis	20
305	0.90	Emesis	4
285	0.90	Emesis	4
355	0.85	Emesis	5
280	0.85	Emesis	8
315	0.85	Emesis	4
360	0.85	Emesis	3
290	0.85	No Emesis	20
335	0.85	Emesis	2
315	0.85	No Emesis	20
325	0.85	Emesis	3
305	0.85	Emesis	12
310	0.85	No Emesis	20
330	0.85	No Emesis	20
290	0.85	Emesis	3
305	0.85	Emesis	4
270	0.85	No Emesis	20
350	0.85	Emesis	8
320	0.85	No Emesis	20
320	0.80	Emesis	15
345	0.80	No Emesis	20
295	0.80	No Emesis	20
325	0.80	No Emesis	20
360	0.80	Emesis	3
275	0.80	No Emesis	20
315	0.80	No Emesis	20
315	0.80	Emesis	2

TABLE 5 b

Summary Table of 5 a. The Average Minimum Emetic Dose of Preparation 104.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90*	12	10	2
0.85	16	10	6
0.80	8	3	5
* Minimum Emetic Dose			

TABLE 6 a

Preparation 104. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
320	0.90	Emesis	9
350	0.90	No Emesis	20
340	0.90	Emesis	7
330	0.90	Emesis	5
290	0.85	Emesis	8
320	0.85	No Emesis	20
338	0.85	Emesis	6
345	0.85	Emesis	4
320	0.80	Emesis	11
325	0.80	Emesis	10
365	0.80	No Emesis	20
300	0.80	Emesis	3
300	0.80	Emesis	14
275	0.80	Emesis	9
320	0.80	Emesis	7
355	0.80	Emesis	2
295	0.75	Emesis	5
375	0.75	Emesis	9
340	0.75	Emesis	7
325	0.75	Emesis	8
340	0.75	No Emesis	20
270	0.75	Emesis	15
340	0.75	Emesis	11
325	0.75	Emesis	3
350	0.75	Emesis	5
305	0.75	Emesis	2
365	0.75	Emesis	2
385	0.75	Emesis	2
295	0.75	Emesis	15
320	0.75	Emesis	7
320	0.75	Emesis	2
330	0.75	Emesis	15
305	0.70	No Emesis	20
305	0.70	No Emesis	20
280	0.70	No Emesis	20
280	0.70	No Emesis	20
355	0.70	No Emesis	20

(Continued next page)

TABLE 6 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
250	0.70	Emesis	14
365	0.70	Emesis	10
290	0.70	Emesis	5
320	0.70	No Emesis	20
330	0.70	Emesis	13
345	0.70	Emesis	10
350	0.70	Emesis	3

TABLE 6 b

Summary of Table 6 a. The Average Minimum Emetic Dose of Preparation 104.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90	4	3	1
0.85	4	3	1
0.80	8	7	1
0.75*	16	15	1
0.70	12	6	6

* Minimum Emetic Dose

TABLE 7 a

Preparation 104. Results of Each Injection to Determine the Average Minimum Anesthetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
345	0.85	Anesias	2
360	0.85	Anesias	7
285	0.85	Anesias	10
370	0.85	Anesias	14
305	0.80	No Anesias	20
315	0.80	Anesias	2
325	0.80	Anesias	15
330	0.80	No Anesias	20
355	0.80	Anesias	6
335	0.80	Anesias	13
330	0.80	Anesias	8
310	0.80	Anesias	2
300	0.80	Anesias	2
310	0.80	Anesias	11
320	0.80	Anesias	10
370	0.80	No Anesias	20
370	0.75	Anesias	2
365	0.75	Anesias	3
355	0.75	Anesias	9
350	0.75	Anesias	5
295	0.75	Anesias	20
350	0.75	Anesias	5
390	0.75	Anesias	9
295	0.75	No Anesias	20
355	0.75	Anesias	15
305	0.75	Anesias	7
300	0.75	No Anesias	20
335	0.75	Anesias	2
315	0.75	No Anesias	20
340	0.75	Anesias	5
310	0.75	Anesias	8
315	0.75	Anesias	10
305	0.75	Anesias	14
295	0.75	Anesias	12
330	0.75	No Anesias	20
280	0.75	Anesias	20

TABLE 7 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
360	0.70	No Emesis	20
305	0.70	Emesis	10
265	0.70	No Emesis	20
345	0.70	No Emesis	20
325	0.70	Emesis	10
260	0.70	Emesis	10
295	0.70	No Emesis	20
300	0.70	Emesis	5
305	0.70	Emesis	8
300	0.70	Emesis	7
325	0.70	Emesis	3
290	0.70	No Emesis	20
245	0.70	No Emesis	20
260	0.70	No Emesis	20
260	0.70	No Emesis	20
295	0.70	Emesis	8
275	0.70	Emesis	9
270	0.70	Emesis	3
280	0.70	Emesis	5
290	0.70	No Emesis	20

TABLE 7 b

Summary of Table 7 a. The Average Minimum Emetic Dose of Preparation 104.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.85	4	4	
0.20	12	9	3
0.75*	20	16	4
0.70	20	11	9

* Minimum Emetic Dose

TABLE 8

Preparation 106. The Average Minimum Emetic Dose as Previously Determined (1).

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.60	4	4	
0.55	4	4	
0.50	4	4	
0.45	4	3	1
0.40	4	3	1
0.35	12	10	2
0.30*	12	11	1
0.25	8	3	5

* Minimum Emetic Dose

TABLE 9 a

Preparation 106. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./KG.	Results	Time, in Minutes, Observed
270	0.40	Emesis	9
285	0.40	Emesis	14
330	0.40	Emesis	15
290	0.40	Emesis	5
310	0.35	No Emesis	20
295	0.35	Emesis	7
275	0.35	Emesis	13
260	0.35	No Emesis	20
300	0.35	Emesis	10
280	0.35	Emesis	7
345	0.35	Emesis	14
280	0.35	Emesis	8
340	0.35	Emesis	20
325	0.35	Emesis	8
295	0.35	Emesis	3
320	0.35	Emesis	6
275	0.35	Emesis	4
295	0.35	Emesis	8
315	0.35	No Emesis	20
340	0.35	Emesis	6
340	0.35	Emesis	11
330	0.35	Emesis	7
305	0.35	No Emesis	20
315	0.35	Emesis	12
325	0.30	No Emesis	20
330	0.30	Emesis	18
270	0.30	Emesis	20
320	0.30	No Emesis	20
305	0.30	No Emesis	20
365	0.30	No Emesis	20
330	0.30	Emesis	10
330	0.30	Emesis	14
315	0.30	No Emesis	20
355	0.30	Emesis	6
345	0.30	Emesis	7
330	0.30	Emesis	6
320	0.30	No Emesis	20

(Continued next page)

TABLE 9 a. (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
355	0.30	Emesis	10
345	0.30	Emesis	15
335	0.30	No Emesis	20
290	0.30	Emesis	14
315	0.30	No Emesis	20
325	0.30	Emesis	13
360	0.30	Emesis	6
265	0.25	No Emesis	20
345	0.25	No Emesis	20
330	0.25	No Emesis	20
370	0.25	No Emesis	20
370	0.25	Emesis	6
310	0.25	No Emesis	20
320	0.25	No Emesis	20
285	0.25	No Emesis	20
320	0.25	No Emesis	20
335	0.25	No Emesis	20
345	0.25	Emesis	6
295	0.25	No Emesis	20

TABLE 9 b

Summary of Table 9 a. The Average Minimum Emetic Dose of Preparation 106.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.40	4	4	
0.35*	20	16	4
0.30	20	12	8
0.25	12	2	10
* Minimum Emetic Dose			

TABLE 10

Preparation 107. The Average Minimum Emetic Dose as Previously Determined (1).

Dose cc./kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90	4	4	
0.80	4	4	
0.75	3	6	2
0.70*	12	9	3
0.65	8	4	4
0.60	4	1	3
* Minimum Emetic Dose			

TABLE 11 a

Preparation 107. Results of Each Injection to Determine the Average Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
265	0.95	Emesis	20
335	0.95	Emesis	14
320	0.95	No Emesis	20
345	0.95	Emesis	6
290	0.95	No Emesis	20
340	0.95	Emesis	5
310	0.95	Emesis	4
280	0.95	Emesis	3
315	0.95	Emesis	5
345	0.95	No Emesis	20
320	0.95	Emesis	12
315	0.95	Emesis	6
380	0.95	Emesis	4
320	0.95	No Emesis	20
345	0.95	No Emesis	20
335	0.95	Emesis	10
320	0.95	Emesis	20
365	0.95	Emesis	20
360	0.95	Emesis	11
335	0.95	Emesis	16
375	0.90	Emesis	5
305	0.90	Emesis	4
345	0.90	Emesis	15
330	0.90	No Emesis	20
285	0.90	Emesis	5
325	0.90	No Emesis	20
295	0.90	Emesis	7
305	0.90	No Emesis	20
260	0.90	Emesis	3
285	0.90	No Emesis	20
345	0.90	Emesis	5
295	0.90	Emesis	6
340	0.90	No Emesis	20
300	0.90	Emesis	5
265	0.90	No Emesis	20
350	0.90	No Emesis	20
355	0.90	No Emesis	20

(Continued next page)

TABLE 11 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
340	0.90	Emesis	14
345	0.90	Emesis	17
310	0.90	No Emesis	20
315	0.85	No Emesis	20
280	0.85	No Emesis	20
285	0.85	No Emesis	20
340	0.85	Emesis	10
340	0.85	Emesis	11
295	0.85	Emesis	15
335	0.85	Emesis	8
350	0.85	Emesis	6
290	0.80	No Emesis	20
310	0.80	Emesis	2
320	0.80	No Emesis	20
320	0.80	No Emesis	20
315	0.80	Emesis	5
305	0.80	Emesis	12
310	0.80	No Emesis	20
350	0.80	No Emesis	20

TABLE 11 b

Summary of Table 11 a. The Average Minimum Emetic Dose of Preparation 107

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.95*	20	15	5
0.90	20	11	9
0.85	8	5	3
0.80	8	3	5

* Minimum Emetic Dose

TABLE 12

Preparation 106. The Average Minimum Emetic Dose as Previously Determined (1).

Dose cc./kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90	4	4	
0.80	3	6	2
0.75*	3	8	
0.70	3	4	4
0.60	4	1	3

* Minimum Emetic Dose

TABLE 13 a

Preparation 108. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
340	0.85	No Emesis	20
315	0.85	Emesis	16
275	0.85	Emesis	4
345	0.85	Emesis	8
320	0.85	Emesis	3
355	0.85	Emesis	9
295	0.85	Emesis	5
355	0.85	Emesis	10
340	0.80	Emesis	11
285	0.80	No Emesis	20
325	0.80	Emesis	8
310	0.80	Emesis	5
295	0.80	Emesis	11
305	0.80	Emesis	4
330	0.80	Emesis	5
350	0.80	Emesis	8
345	0.80	No Emesis	20
365	0.80	No Emesis	20
320	0.80	Emesis	5
290	0.80	No Emesis	20
300	0.80	Emesis	5
340	0.80	Emesis	7
300	0.80	Emesis	8
275	0.80	No Emesis	20
355	0.80	Emesis	10
370	0.80	Emesis	13
320	0.80	Emesis	9
330	0.80	Emesis	4
280	0.75	No Emesis	20
325	0.75	Emesis	4
290	0.75	Emesis	5
330	0.75	No Emesis	20
285	0.75	Emesis	10
295	0.75	Emesis	20
305	0.75	Emesis	10
340	0.75	Emesis	15
335	0.75	Emesis	15

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TABLE 13 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
290	0.75	No Emesis	20
310	0.75	No Emesis	20
320	0.75	No Emesis	20
340	0.75	No Emesis	20
335	0.75	No Emesis	20
330	0.75	Emesis	11
325	0.75	Emesis	18
290	0.75	No Emesis	20
330	0.75	Emesis	10
390	0.75	Emesis	10
310	0.75	No Emesis	20
295	0.70	Emesis	4
260	0.70	Emesis	10
270	0.70	Emesis	4
330	0.70	No Emesis	20
275	0.70	No Emesis	20
310	0.70	Emesis	11
375	0.70	Emesis	3
360	0.70	No Emesis	20
330	0.70	No Emesis	20
355	0.70	Emesis	20
380	0.70	No Emesis	20
325	0.70	No Emesis	20

TABLE 13 b

Summary of Table 13 a. The Average Minimum Emetic Dose of Preparation 108.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.85	8	7	1
0.80*	20	15	5
0.75	20	11	9
0.70	12	6	6

* Minimum Emetic Dose

TABLE 14 a

Preparation 108. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
300	0.80	Emesis	8
245	0.80	Emesis	2
325	0.80	Emesis	2
230	0.80	Emesis	7
290	0.80	No Emesis	20
300	0.80	Emesis	15
275	0.80	No Emesis	20
285	0.80	No Emesis	20
295	0.80	Emesis	3
320	0.80	Emesis	20
325	0.80	No Emesis	20
320	0.80	Emesis	7
275	0.80	Emesis	12
310	0.80	Emesis	5
355	0.80	Emesis	4
360	0.80	Emesis	4
320	0.80	Emesis	6
335	0.80	Emesis	13
305	0.80	Emesis	15
330	0.80	Emesis	11
295	0.75	Emesis	6
310	0.75	Emesis	3
355	0.75	No Emesis	20
370	0.75	Emesis	7
255	0.75	No Emesis	20
265	0.75	No Emesis	20
270	0.75	Emesis	10
290	0.75	No Emesis	20
295	0.75	Emesis	7
290	0.75	No Emesis	20
335	0.75	No Emesis	20
335	0.75	Emesis	6
320	0.75	Emesis	7
300	0.75	Emesis	8
280	0.75	Emesis	9
280	0.75	Emesis	8
340	0.70	Emesis	9

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TABLE 14 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
275	0.70	No Emesis	20
295	0.70	No Emesis	20
325	0.70	Emesis	6
300	0.70	Emesis	9
325	0.70	Emesis	8
295	0.70	No Emesis	20
340	0.70	No Emesis	20
240	0.70	No Emesis	20
300	0.70	No Emesis	20
290	0.70	Emesis	4
285	0.70	No Emesis	20
290	0.65	No Emesis	20
295	0.65	No Emesis	20
355	0.65	No Emesis	20
330	0.65	No Emesis	20

TABLE 14 b

Summary of Table 14 a. The Average Minimum Emetic Dose of Preparation 108.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.80*	20	16	4
0.75	16	10	6
0.70	12	5	7
0.65	4		4

* Minimum Emetic Dose

TABLE 15 a

Preparation 110. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
345	0.65	Emesis	6
355	0.65	Emesis	3
330	0.65	Emesis	2
330	0.65	Emesis	6
315	0.65	Emesis	5
330	0.65	Emesis	12
300	0.65	No Emesis	20
280	0.65	Emesis	7
325	0.60	Emesis	3
345	0.60	Emesis	9
295	0.60	Emesis	10
310	0.60	Emesis	8
340	0.60	Emesis	11
400	0.60	Emesis	10
290	0.60	Emesis	17
320	0.60	Emesis	15
340	0.60	Emesis	20
365	0.60	Emesis	12
300	0.60	No Emesis	20
375	0.60	No Emesis	20
325	0.60	Emesis	13
270	0.60	Emesis	12
350	0.60	Emesis	4
320	0.60	Emesis	17
300	0.60	Emesis	3
315	0.60	Emesis	6
310	0.60	Emesis	6
310	0.60	Emesis	4
325	0.55	No Emesis	20
285	0.55	Emesis	8
345	0.55	Emesis	5
315	0.55	Emesis	2
300	0.55	No Emesis	20
285	0.55	Emesis	6
345	0.55	Emesis	4
270	0.55	No Emesis	20
315	0.65	Emesis	6

(Continued next page)

TABLE 15 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
315	0.55	No Emesis	20
340	0.55	Emesis	10
285	0.55	No Emesis	20
320	0.55	Emesis	4
355	0.55	Emesis	5
355	0.55	Emesis	12
345	0.55	Emesis	7
335	0.55	No Emesis	20
365	0.55	Emesis	7
335	0.55	Emesis	8
340	0.55	No Emesis	20
350	0.50	No Emesis	20
300	0.50	No Emesis	20
285	0.50	No Emesis	20
350	0.50	Emesis	10
270	0.50	No Emesis	20
275	0.50	No Emesis	20
350	0.50	No Emesis	20
355	0.50	Emesis	8
355	0.50	Emesis	8
285	0.50	Emesis	8
370	0.50	Emesis	7
340	0.50	No Emesis	20
370	0.50	Emesis	7
375	0.50	Emesis	8
370	0.50	Emesis	10
335	0.50	No Emesis	20
355	0.50	No Emesis	20
295	0.50	Emesis	11
320	0.50	No Emesis	20
310	0.50	Emesis	10
345	0.45	No Emesis	20
335	0.45	No Emesis	20
335	0.45	Emesis	14
305	0.45	No Emesis	20
320	0.45	Emesis	6
285	0.45	Emesis	11
350	0.45	Emesis	20
335	0.45	No Emesis	20

TABLE 15 b

Summary of Table 15 a. The Average Minimum Emetic Dose of Preparation 110.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.65	8	7	1
0.60*	20	18	2
0.55	20	13	7
0.50	20	10	10
0.45	8	4	4
* Minimum Emetic Dose			

TABLE 16 a

Preparation 110. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
310	0.65	Emesis	4
360	0.65	Emesis	6
325	0.65	Emesis	9
350	0.65	Emesis	10
350	0.65	Emesis	12
340	0.65	Emesis	6
325	0.65	No Emesis	20
225	0.65	No Emesis	20
275	0.65	Emesis	8
280	0.65	Emesis	4
285	0.65	Emesis	10
290	0.65	Emesis	9
220	0.65	No Emesis	20
295	0.65	Emesis	10
325	0.65	Emesis	8
330	0.65	Emesis	15
335	0.65	Emesis	2
340	0.65	Emesis	13
310	0.60	No Emesis	20
250	0.60	No Emesis	20
270	0.60	Emesis	20
345	0.60	Emesis	5
355	0.60	Emesis	7
345	0.60	Emesis	4
335	0.60	Emesis	6
335	0.60	Emesis	15
340	0.60	No Emesis	20
340	0.60	Emesis	6
325	0.60	No Emesis	20
290	0.60	Emesis	8
330	0.60	No Emesis	20
375	0.60	Emesis	10
295	0.60	Emesis	6
370	0.60	Emesis	11
370	0.60	Emesis	4
350	0.60	Emesis	7
325	0.60	Emesis	9

(Continued next page)

TABLE 16 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
305	0.60	No Emesis	20
355	0.55	Emesis	9
290	0.55	No Emesis	20
390	0.55	Emesis	9
300	0.55	Emesis	4
380	0.55	Emesis	5
385	0.55	No Emesis	20
395	0.55	Emesis	3
340	0.55	No Emesis	20
345	0.55	No Emesis	20
340	0.55	Emesis	7
335	0.55	Emesis	3
385	0.55	No Emesis	20
345	0.55	Emesis	12
345	0.55	Emesis	18
310	0.55	No Emesis	20
340	0.55	Emesis	15
320	0.55	Emesis	4
320	0.55	Emesis	3
280	0.55	No Emesis	20
325	0.55	Emesis	4
315	0.50	No Emesis	20
345	0.50	Emesis	8
285	0.50	Emesis	13
300	0.50	No Emesis	20
340	0.50	Emesis	7
295	0.50	Emesis	3
365	0.50	Emesis	5
370	0.50	Emesis	11
295	0.50	No Emesis	20
310	0.50	Emesis	9
380	0.50	Emesis	8
355	0.50	Emesis	14
350	0.50	Emesis	11
360	0.50	Emesis	13
315	0.50	No Emesis	20
320	0.50	Emesis	9
315	0.50	Emesis	9
300	0.50	No Emesis	20
330	0.50	Emesis	12

(Continued next page)

TABLE 16 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
345	0.50	No Emesis	20
350	0.30	No Emesis	20
310	0.30	No Emesis	20
365	0.30	No Emesis	20
335	0.30	No Emesis	20

TABLE 16 b

Summary of Table 16 a. The Average Minimum Emetic Dose of Preparation 110.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.65*	18	15	3
0.60	20	14	6
0.55	20	13	7
0.50	20	14	6
0.30	4		4

* Minimum Emetic Dose

TABLE 17 a

Preparation 114. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
345	0.90	Emesis	12
265	0.90	Emesis	4
300	0.90	Emesis	9
290	0.90	Emesis	6
296	0.85	Emesis	3
295	0.85	Emesis	6
280	0.85	Emesis	17
295	0.85	Emesis	7
310	0.85	Emesis	6
290	0.85	Emesis	5
285	0.85	Emesis	9
255	0.85	Emesis	6
285	0.85	Emesis	4
315	0.85	Emesis	4
300	0.85	Emesis	4
255	0.85	Emesis	9
350	0.85	Emesis	7
325	0.85	Emesis	3
325	0.85	Emesis	12
305	0.80	No Emesis	20
305	0.80	No Emesis	20
355	0.80	No Emesis	20
320	0.80	Emesis	8
295	0.80	Emesis	2
290	0.80	Emesis	6
270	0.80	Emesis	14
255	0.80	No Emesis	20
265	0.80	Emesis	5
270	0.80	Emesis	3
330	0.80	Emesis	3
310	0.80	Emesis	10
295	0.80	No Emesis	20
260	0.80	Emesis	5
280	0.80	Emesis	4
255	0.80	No Emesis	20
300	0.80	Emesis	9
300	0.80	Emesis	4

(Continued next page)

TABLE 17 a (Continued)

Weight of Pigeon in Grams	Dose cc./Kg.	Results	Time, in Minutes, Observed
260	0.80	No Emesis	20
285	0.80	Emesis	7
270	0.75	Emesis	10
285	0.75	Emesis	4
300	0.75	No Emesis	20
280	0.75	Emesis	9
240	0.75	No Emesis	20
270	0.75	No Emesis	20
295	0.75	Emesis	5
335	0.75	Emesis	2
295	0.75	Emesis	6
280	0.75	No Emesis	20
295	0.75	Emesis	5
320	0.75	Emesis	8
335	0.75	No Emesis	20
360	0.75	Emesis	10
290	0.75	No Emesis	20
290	0.75	Emesis	10
295	0.75	Emesis	7
275	0.75	Emesis	6
295	0.75	Emesis	2
275	0.75	Emesis	7
265	0.70	No Emesis	20
300	0.70	No Emesis	20
345	0.70	No Emesis	20
285	0.70	Emesis	6
285	0.70	No Emesis	20
330	0.70	Emesis	7
335	0.70	Emesis	4
260	0.70	No Emesis	20

TABLE 17 b

Summary of Table 17 a. The Average Minimum Emetic Dose of Preparation 114.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.90	4	4	
0.85*	15	15	
0.80	20	13	7
0.75	20	14	6
0.70	8	3	5
* Minimum Emetic Dose			

TABLE 18 a

Preparation 116. Results of Each Injection to Determine the Average Minimum Emetic Dose.

Weight of Pigeon in Grams.	Dose cc./Kg.	Results	Time, in Minutes, Observed
300	0.60	Emesis	11
335	0.60	Emesis	10
315	0.60	Emesis	9
310	0.60	No Emesis	20
280	0.60	Emesis	11
370	0.60	No Emesis	20
325	0.60	Emesis	6
345	0.60	Emesis	11
350	0.55	Emesis	10
315	0.55	Emesis	9
320	0.55	Emesis	6
365	0.55	Emesis	4
330	0.55	Emesis	6
365	0.55	No Emesis	20
350	0.55	No Emesis	20
310	0.55	Emesis	7
345	0.55	Emesis	5
310	0.55	Emesis	9
340	0.55	Emesis	12
385	0.55	Emesis	9
345	0.55	Emesis	7
380	0.55	No Emesis	20
325	0.55	No Emesis	20
315	0.55	Emesis	10
290	0.50	Emesis	6
320	0.50	Emesis	2
335	0.50	No Emesis	20
320	0.50	Emesis	4
270	0.50	No Emesis	20
295	0.50	Emesis	5
350	0.50	Emesis	4
290	0.50	Emesis	14
335	0.50	No Emesis	20
320	0.50	Emesis	2
350	0.50	Emesis	15
290	0.50	No Emesis	20
375	0.45	Emesis	3

(Continued next page)

TABLE 18 a (Continued)

Weight of Pigeon in Grams.	Dose cc./Kg.	Results	Time, in Minutes, Observed
315	0.45	No Emesis	20
290	0.45	No Emesis	20
345	0.45	No Emesis	20
290	0.45	No Emesis	20
330	0.45	No Emesis	20
360	0.45	No Emesis	20
290	0.45	Emesis	7

TABLE 18 b

Summary of Table 18 a. The average Minimum Emetic Dose of Preparation 116.

Dose cc./Kg.	Number of Injections	Results of Injections	
		Emesis	No Emesis
0.60	8	6	2
0.55*	16	12	4
0.50	12	8	4
0.45	8	2	6

* Minimum Emetic Dose

TABLE 19

Summary of Tables 2 to 18. A Comparison of the M.E.m.D. of the Eight Tinctures Showing the Redeterminations.

Preparation	M.E.m.D. Previous (1) Determination	M.E.m.D. This Report	M.E.m.D. Second Determination	M.E.m.D. Third Determination
102	0.50	0.45		
104	0.90	0.90	0.75	0.75
106	0.30	0.35		
107	0.70	0.95		
108	0.75	0.80	0.80	
110		0.60	0.65	
114		0.85		
116		0.55		

DISCUSSION

One question to be considered with regard to the five preparations that were used in the previous investigation is that of deterioration. By referring to Table 19 it may be seen that only one preparation (107) shows enough difference between previous determinations and the present ones to warrant consideration of deterioration. Since the difference here is so much larger than with the other preparations it is indicated that Preparation 107 has lost part of its activity. The fact that the tincture contained a sediment which did not go into solution on shaking, further indicates that deterioration may have taken place.

It may be said concerning the number of injections for each dose that 12 seems to be a sufficient number to determine the action of a specific dose. In most cases throughout this investigation 20 injections per dose were used. In one preparation 32 injections per dose were made. However, there was only one preparation (108) in which the re-

sults of the first 12 injections of each dose would alter the M.E.M.D. as determined by the 20 injections. Burn (6) recommended that 25 pigeons be used for each dose in standardizing digitalis, but from the results obtained in this study it seems that 12 injections per dose will give the reaction of that dose.

ACCURACY OF THE METHOD

The size of the doses given has been varied by 0.05 cc. per Kg. This is about as small a difference as can be measured using the dilutions necessary and a syringe graduated to 1/100 cc. The difference in this dose can be detected easily as is evidenced by the difference in number of pigeons reacting. In the 17 standardizations it may be seen by comparing the M.E.M.D. and the dose 0.05 cc. per Kg. smaller, that for the dose just smaller the range of positive reactions is from 35 per cent to 70 per cent, with an average of 55 per cent and with the M.E.M.D. the range is from 75 to 100 per cent positives, with an average of 83 per cent. Thus it is seen that the difference of 0.05 cc. per Kg. can be detected easily. This difference of 0.05 cc. per Kg. makes a difference in size of dose ranging from 5.25 to 16.7 per cent, depending upon the size of the M.E.M.D. From the standpoint in variance of size of dose the method is measuring to within 5 to 16 per cent. However, there still remained the question as to whether a second determination would give results comparable to the first.

In order to settle the question of how close a second determination would approach the first, redeterminations were made with three titrations and a third determination was made with one of these. Also the

M.E.D. of the five tinctures from the previous study may be compared. Table 19 shows these differences and it may be seen that in no case is the difference greater than 16.7 per cent except in Preparation 107. As mentioned above, the indications are that there was deterioration in this tincture. Since no difference is in excess of 20 per cent, it may be said that the method is accurate to within 5 to 20 per cent, which is a very good accuracy for a biological assay.

Throughout this investigation Preparation 104 has been looked upon as the standard because it was obtained from a mixture of five samples of the crude drug. It seems that the M.E.D. of this tincture might serve as a standard since it does represent a composite sample. It would be well to allow 10 per cent either way, which would place the dose within the degree of accuracy. Thus it may be stated that Tincture of Gelsemium should have a Minimum Emetic Dose of 0.75 cc. per Kg., plus or minus 10 per cent.

SENSITIVENESS OF PIGEONS

Several workers who have used pigeons in assaying other drugs have referred to the possibility of the animal's becoming more sensitive when used for several successive administrations. Various periods of time between injections, varying from one week to one month, have been recommended to prevent the occurrence of sensitiveness. Riddle and Burns (7) report that in administering oral doses of yohimbine hydrochloride the pigeons become so sensitive that the mere entrance of the worker into the cage provokes emesis. Lieb and Mulinos (8) reported a spontaneous emesis with pigeons receiving injections of digitalis and they state that a

sensitiveness is acquired only when the routine of administration is strictly observed. That is, should the period of starvation, for instance, be left off, the pigeon will not show the sensitiveness.

In the present study there was noticed a few instances of spontaneous emesis. On one day 5 birds which had been prepared for injection in the usual manner were noticed to vomit before any injection was made. On two subsequent days 1 bird was noticed to vomit prior to an injection. The fact that 1 of these 7 was found dead a few days after first noticing its peculiar reaction indicates that these probably were not normal healthy birds. Because of this spontaneous emesis it was decided to make a study to determine whether or not the birds as a group were becoming more sensitive to doses of the drug when allowed the usual resting period of 2 weeks between injections.

On the day that the pigeons were noticed to vomit with no injections they were injected with a solution of the same alcoholic strength as that of the tinctures used. This was diluted with saline solution in the same manner that the tincture is diluted before injection. There was emesis in 3 of the 7 birds. After an elapse of about 6 weeks the 6 birds (1 having died, as mentioned above) were injected with a dose of a tincture which was a little larger than half the M.Em.D. of that tincture. Emesis was produced in 5 of the 6. A month later the 6 were injected with the alcoholic saline solution and emesis was produced in only 1. After a rest of 2 weeks they were again injected with the alcoholic saline solution and no emesis was obtained. After another rest they were injected with a dose of a tincture equivalent to half its M.Em.D. and emesis was obtained in only two. This indicates that if a

pigeon does become more sensitive it can be desensitized by rest periods and saline injections. Lieb and Mulinos (8) found in their work that the pigeon could be desensitized by saline injections. This also appears to be true when they become sensitive to Gelsemium.

In order to test the group for sensitiveness each of the 85 birds used in this study was injected with a dose of tincture which was slightly over half the M.Ed.D. There was emesis in 15 of the birds which is about 17 per cent. These 15 were removed from routine use in standardising preparations. After an elapse of 2 weeks they were again injected with the same dose of the same preparation and 9 of them vomited. After another 2 weeks the process was repeated and again 9 vomited, but some that reacted before did not react this time. The birds were then injected with alcoholic saline solution and only one reaction was obtained. Upon injecting with alcoholic saline solution after the customary rest period no reactions were obtained. This lack of reaction indicates that the sensitiveness can be overcome.

After the removal from the group of these more sensitive birds several preparations were standardised again to determine whether or not the group as a whole would show any difference in reaction from that shown before the question of sensitiveness was considered. As can be seen by comparing Tables 6 and 7 with 5, Table 14 with 13, and Table 16 with 15 there was very little difference in the M.Ed.D. In one case it was the same and in another it was 0.05 cc./Kg. higher after the pigeons had been used for a number of injections than it was when the birds were first used. In the case of Preparation 104 (Tables 5, 6 and 7) the

M.E.M.D. was about 16.5 per cent smaller in the later determinations, but, as is mentioned in another place in this work, this is felt to be within experimental error.

Because of the data as given above it is not felt that as a group the pigeons become any more sensitive to the drug than the limit of experimental error. It is suggested that it would be advisable in using the method to make preliminary tests in order to determine if any of the pigeons are hypersensitive, and that if any prove so, they be discarded.

The few birds which might show a spontaneous emesis will not impair the usefulness of the method, for in all probability they would be eliminated in the preliminary tests. Should a bird develop a sensitiveness during use, it would be easy for the worker to notice and eliminate it. It is felt from the large number of injections (about two thousand) that have been made in this study that the number of birds becoming hypersensitive during repeated use will be so small that final results will not be noticeably affected. A two weeks rest period was maintained throughout the study, but other workers (8) have suggested a month be allowed. While it is not felt that such a length of time is necessary, it might have served to prevent the development of sensitiveness in the few that did prove more sensitive.

OTHER METHODS OF BIOLOGICAL ASSAY

MINIMUM LETHAL DOSE FOR FROGS

Since there was no data available showing the M.L.D. of tinctures of Gelsemium for frogs it was thought that the determination of this would be of interest in the present study for comparative purposes. Preliminary investigations were made using several tinctures. The tincture was evaporated at room temperature and made up to three fifths its original volume in order to concentrate and to reduce the alcoholic strength for injection. Injection was made into the ventral lymph sac.

During these investigations the following observations were made: Within two or three minutes after injection the frog became relaxed, with no effort to turn over when placed upon its back. At the end of one hour some frogs showed no apparent signs of life but when opened their hearts were found beating and they would continue to beat for three or more hours after injection. When stopped some of the hearts were found in systole and some in diastole. Other frogs receiving larger doses of tincture were left undisturbed for twenty-four hours and they recovered. Thus it may be seen that the time of death is very difficult to determine. For that reason a twenty-four hour observation period was adopted.

The M.L.D. for three of the tinctures was determined. Twelve injections per dose within the critical range were made and the M.L.D. taken as that dose killing 9 out of 12 frogs. The doses were varied by 0.002 cc./Gm. The M.L.D. for the 3 tinctures was found to be: for Preparation 106, 0.018 cc./Gm.; Preparation 107, 0.038; and for Prepara-

tion 108, 0.038 cc./Gm. In comparing these M.L.D.'s. to the M.E.M.D.'s. of these same tinctures it is seen that they run somewhat parallel, however, the number of preparations is inadequate for drawing conclusions.

After determining the M.L.D. for these three preparations it was decided that the method was too unsatisfactory for use because of the indefinite end point and the difficulty involved in injecting frogs and holding them for twenty-four hours for observation, so no further determinations were made.

MINIMUM LETHAL DOSE FOR MICE

Swanson and Hargreaves (9) recommended the determination of M.L.D. for mice as a means of biological assay for Gelsemium preparations. It was deemed desirable to use this method of assay in order that the M.L.D. might be compared with the M.E.M.D.

Injections were made intraperitoneally using a 1 cc. syringe graduated in hundredths and a 26 gauge half inch needle. Tinctures were diluted 1 part tincture to 19 parts physiological saline solution or 1 part tincture to 9 parts saline solution, depending upon the strength of the tincture. Tables 20 to 26, inclusive, show the results of the injections, and the "b" tables show the summary of results and the M.L.D. The Minimum Lethal Dose is defined as that dose measured in cc. per gram which will kill three fourths of the injected mice within one hour. Many workers use 50 per cent deaths as the end point but due to the fact that 75 per cent emesis was chosen as the M.E.M.D. it was decided that 75 per cent deaths would give figures which could be compared more favorably. Twelve injections per dose were made within the range of the

M.L.D., which seemed adequate to show the effect of the dose. The dose was expressed in cc. per gram and was varied by 0.00005 cc. per gram. This variance between doses gave results which were easily distinguishable.

The mice died from respiratory failure in from twenty to thirty minutes after injection. Convulsions were pronounced and a mouse seldom survived after they became apparent. On opening some, the heart would be found beating after respiration had ceased. Of the nearly four hundred injections made about eighty-five mice were opened after death. The majority of the hearts were in ventricular systole, in fact only 15 were in diastole and of these 15 several seemed to have the right ventricle dilated and the left ventricle contracted. If the dose were not fatal, recovery was rapid, for in no case did death occur after an hour and the mouse seemed completely recovered after this time.

By referring to Table 27 the comparison with the M.E.M.D. may be seen. There is rather close parallelism in the M.E.M.D. and the M.L.D. Preparation 104 is used as the standard. By referring the remaining six preparations to this it can be seen that in the majority of the tinctures there is about the same per cent of range between the M.L.D.'s. as there is between the M.E.M.D.'s. of the same preparations. In two tinctures, numbers 110 and 114, this relationship is not as close as in the other preparations, but even in these the difference is not so very great. The above data seems to show that there is some relationship between toxicity and the emetic action and further indicates that this emetic action does measure the activity of the drug. The average ratio between the M.E.M.D. and the M.L.D. for mice is 1:2.1, on the basis of Kg. of each.

TABLE 20 a

Preparation 102. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
18.5	0.00130	Death
17.0	0.00130	Death
21.0	0.00130	Death
20.0	0.00130	Death
19.5	0.00120	Death
18.0	0.00120	Death
18.0	0.00120	Death
17.5	0.00120	Death
20.0	0.00100	Death
17.5	0.00100	Recovery
19.5	0.00100	Death
19.5	0.00100	Death
18.0	0.00090	Death
20.5	0.00090	Death
21.0	0.00090	Death
21.5	0.00090	Death
20.0	0.00080	Death
19.0	0.00080	Death
18.5	0.00080	Death
20.5	0.00080	Death
19.5	0.00080	Death
18.0	0.00080	Death
18.5	0.00080	Death
18.0	0.00080	Recovery
19.0	0.00075	Death
19.5	0.00075	Recovery
23.0	0.00075	Recovery
17.0	0.00075	Death
16.5	0.00075	Death
18.5	0.00075	Death
18.5	0.00075	Death
21.0	0.00075	Death
20.0	0.00075	Recovery
21.0	0.00075	Death
20.5	0.00075	Death
20.0	0.00075	Death
17.0	0.00070	Death

(Continued next page)

TABLE 20 a (Continued)

Weight of Mouse in Grams	Dose cc./Gm.	Results after One Hour Observation
17.5	0.00070	Recovery
18.5	0.00070	Death
19.0	0.00070	Death
17.0	0.00070	Recovery
17.0	0.00070	Recovery
19.0	0.00070	Death
19.5	0.00070	Death
18.5	0.00070	Death
19.0	0.00070	Recovery
17.5	0.00070	Recovery
18.0	0.00070	Death
19.5	0.00065	Death
17.5	0.00065	Death
20.0	0.00065	Death
16.5	0.00065	Death
18.5	0.00065	Recovery
17.0	0.00065	Recovery
18.0	0.00065	Recovery
16.0	0.00065	Death
19.5	0.00060	Recovery
19.0	0.00060	Recovery
21.0	0.00060	Recovery
21.5	0.00060	Death
16.5	0.00060	Recovery
16.5	0.00060	Recovery
16.0	0.00060	Recovery
19.0	0.00060	Recovery
20.0	0.00040	Recovery
20.0	0.00040	Recovery
19.0	0.00040	Recovery
19.5	0.00040	Recovery

TABLE 20 b

Summary of Table 20 a. The Minimum Lethal Dose of Preparation 102.

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00130	4	4	
0.00120	4	4	
0.00100	4	3	1
0.00090	4	4	
0.00080	8	7	1
0.00075*	12	9	3
0.00070	12	7	5
0.00065	8	5	3
0.00060	8	1	7
0.00040	4		4
* Minimum Lethal Dose			

TABLE 21 a

Preparation 104. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
17.0	0.00200	Death
16.5	0.00200	Death
17.0	0.00180	Death
16.5	0.00180	Death
18.5	0.00170	Death
14.5	0.00170	Death
16.0	0.00160	Death
18.0	0.00160	Death
17.5	0.00160	Death
20.0	0.00160	Death
16.5	0.00155	Death
17.5	0.00155	Death
21.0	0.00155	Death
16.5	0.00155	Recovery
20.0	0.00155	Death
19.5	0.00155	Recovery
17.5	0.00155	Death
13.5	0.00155	Death
21.5	0.00155	Death
19.0	0.00155	Death
20.5	0.00155	Recovery
16.0	0.00155	Death
21.0	0.00150	Recovery
17.5	0.00150	Death
18.0	0.00150	Recovery
21.0	0.00150	Recovery
24.5	0.00150	Recovery
20.0	0.00150	Death
18.0	0.00150	Death
19.5	0.00150	Recovery
20.0	0.00150	Recovery
20.5	0.00150	Recovery
21.0	0.00150	Recovery
16.5	0.00150	Recovery
18.5	0.00100	Recovery
17.0	0.00100	Recovery
17.0	0.00080	Recovery

(Continued next page)

TABLE 21 a (Continued)

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
16.0	0.00080	Recovery
14.5	0.00080	Recovery
16.0	0.00080	Recovery
16.5	0.00080	Recovery
17.0	0.00080	Recovery
18.5	0.00080	Recovery
17.5	0.00080	Recovery
17.0	0.00050	Recovery
15.5	0.00050	Recovery

TABLE 21 b

Summary of Table 21 a. The Minimum Lethal Dose of Preparation 104.

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00200	2	2	
0.00180	2	2	
0.00170	2	2	
0.00160	4	4	
0.00155*	12	9	3
0.00150	12	3	9
0.00100	2		2
0.00080	4		4
0.00060	4		4
0.00050	2		2

* Minimum Lethal Dose

TABLE 22 a

Preparation 106. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
20.0	0.00060	Death
16.5	0.00060	Death
20.0	0.00060	Death
18.5	0.00060	Death
17.0	0.00060	Death
13.5	0.00060	Death
17.0	0.00060	Death
17.0	0.00060	Death
19.5	0.00055	Recovery
18.0	0.00055	Recovery
19.5	0.00055	Death
17.0	0.00055	Recovery
17.0	0.00055	Death
18.5	0.00055	Death
18.5	0.00055	Death
18.0	0.00055	Recovery
16.5	0.00055	Death
17.0	0.00055	Recovery
20.5	0.00055	Recovery
15.5	0.00055	Recovery
19.5	0.00050	Death
21.0	0.00050	Recovery
17.0	0.00050	Recovery
18.5	0.00050	Death
17.5	0.00050	Recovery
19.5	0.00050	Recovery
16.0	0.00050	Death
17.5	0.00050	Recovery
16.5	0.00045	Death
18.0	0.00045	Recovery
17.0	0.00045	Death
22.0	0.00045	Recovery

TABLE 22 b

Summary of Table 22 a. The Minimum Lethal Dose of Preparation 106.

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00060*	8	8	
0.00055	12	5	7
0.00050	8	3	5
0.00045	4	2	2

* Minimum Lethal Dose

TABLE 23 a

Preparation 107. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
20.5	0.00230	Death
24.5	0.00230	Death
18.0	0.00230	Death
27.0	0.00230	Death
19.5	0.00230	Death
18.5	0.00230	Death
23.5	0.00230	Death
21.5	0.00230	Recovery
20.5	0.00230	Death
26.0	0.00230	Death
17.0	0.00230	Death
15.0	0.00230	Death
24.5	0.00225	Death
15.5	0.00225	Recovery
16.5	0.00225	Death
16.0	0.00225	Death
19.5	0.00225	Death
19.0	0.00225	Death
21.5	0.00225	Recovery
19.5	0.00225	Recovery
22.5	0.00225	Death
20.0	0.00225	Recovery
19.0	0.00225	Recovery
15.0	0.00225	Death
20.5	0.00220	Recovery
18.0	0.00220	Death
15.5	0.00220	Recovery
15.0	0.00220	Death
20.5	0.00220	Death
18.5	0.00220	Recovery
20.0	0.00220	Death
17.5	0.00220	Recovery
25.5	0.00210	Recovery
22.0	0.00210	Recovery
22.0	0.00210	Death
21.0	0.00210	Recovery
14.5	0.00210	Recovery
21.5	0.00210	Recovery
26.0	0.00205	Recovery
19.0	0.00205	Recovery

(Continued next page)

TABLE 23 a (Continued)

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
23.5	0.00205	Recovery
25.5	0.00205	Recovery
20.0	0.00205	Recovery
17.0	0.00205	Death
20.0	0.00200	Death
21.5	0.00200	Recovery
21.5	0.00200	Death
20.5	0.00200	Death
20.0	0.00200	Death
18.5	0.00200	Recovery
17.5	0.00200	Recovery
23.0	0.00200	Recovery
22.0	0.00200	Recovery
23.0	0.00200	Death
19.0	0.00195	Death
21.0	0.00195	Recovery
21.0	0.00195	Recovery
22.5	0.00195	Death
16.5	0.00195	Recovery
17.5	0.00195	Death
20.5	0.00180	Recovery
20.5	0.00180	Recovery
17.5	0.00180	Death
20.0	0.00180	Death
26.5	0.00180	Recovery
24.5	0.00180	Recovery
18.5	0.00170	Recovery
20.0	0.00170	Recovery
26.5	0.00170	Recovery
16.5	0.00170	Recovery
17.5	0.00150	Recovery
20.5	0.00150	Death
18.0	0.00150	Recovery
19.5	0.00150	Death
21.0	0.00140	Death
21.5	0.00140	Death
20.0	0.00140	Recovery
20.0	0.00140	Recovery

TABLE 23 b

Summary of Table 23 a. The Minimum Lethal Dose of Preparation 107

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00230*	12	11	1
0.00225	12	7	5
0.00220	8	4	4
0.00210	6	1	5
0.00205	6	1	5
0.00200	10	5	5
0.00195	6	3	3
0.00180	6	2	4
0.00170	4		4
0.00150	4	2	2
0.00140	4	2	2
* Minimum Lethal Dose			

TABLE 24 a

Preparation 108. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./gm.	Results After One Hour Observation
22.5	0.00180	Death
15.5	0.00180	Death
19.5	0.00180	Death
19.0	0.00180	Death
27.5	0.00175	Recovery
21.5	0.00175	Death
25.0	0.00175	Death
16.0	0.00175	Death
28.0	0.00170	Death
25.0	0.00170	Death
24.0	0.00170	Recovery
18.5	0.00170	Death
20.0	0.00170	Death
20.0	0.00170	Death
19.5	0.00170	Death
16.5	0.00170	Death
20.0	0.00170	Death
16.0	0.00170	Death
19.0	0.00170	Death
20.5	0.00170	Death
22.5	0.00165	Recovery
18.5	0.00165	Recovery
16.0	0.00165	Death
22.0	0.00165	Death
18.5	0.00165	Death
17.5	0.00165	Death
18.0	0.00165	Death
17.0	0.00165	Death
20.5	0.00165	Death
19.5	0.00165	Death
20.0	0.00165	Death
19.0	0.00165	Death
19.5	0.00160	Recovery
21.5	0.00160	Death
21.5	0.00160	Recovery
20.0	0.00160	Recovery
19.5	0.00160	Recovery

(Continued next page)

TABLE 24 a (Continued)

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
20.0	0.00180	Recovery
21.0	0.00180	Recovery
22.0	0.00180	Recovery
18.5	0.00180	Recovery
20.0	0.00180	Recovery
18.0	0.00180	Recovery
19.5	0.00180	Death
20.0	0.00155	Recovery
23.0	0.00155	Recovery

TABLE 24 b

Summary of Table 24 a. The Minimum Lethal Dose for Preparation 108.

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00180	4	4	
0.00175	4	3	1
0.00170	12	11	1
0.00165*	12	10	2
0.00160	12	2	10
0.00155	2		2

* Minimum Lethal Dose

TABLE 25 a

Preparation 110. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
20.5	0.00170	Death
18.5	0.00170	Death
20.0	0.00170	Recovery
18.0	0.00170	Death
18.0	0.00170	Death
22.5	0.00170	Death
21.5	0.00165	Death
16.5	0.00165	Death
19.5	0.00165	Death
21.0	0.00165	Death
18.0	0.00165	Recovery
19.5	0.00165	Death
20.0	0.00165	Death
20.5	0.00165	Death
22.0	0.00165	Death
22.0	0.00165	Death
22.5	0.00165	Death
21.0	0.00165	Death
19.5	0.00160	Recovery
17.5	0.00160	Recovery
23.0	0.00160	Death
20.5	0.00160	Death
20.5	0.00160	Recovery
20.0	0.00160	Death
23.0	0.00160	Recovery
20.0	0.00160	Death
20.5	0.00130	Death
16.5	0.00160	Recovery
18.5	0.00160	Death
20.5	0.00160	Death
16.5	0.00150	Recovery
19.5	0.00150	Recovery
15.0	0.00120	Recovery
15.5	0.00120	Recovery
21.5	0.00100	Recovery
15.0	0.00100	Recovery

TABLE 25 b

Summary of Table 25 a. The Minimum Lethal Dose of Preparation 110

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00170	6	5	1
0.00165*	12	11	1
0.00160	12	7	5
0.00150	2		2
0.00120	2		2
0.00100	2		2
* Minimum Lethal Dose			

TABLE 26 a

Preparation 114. Results of Each Injection to Determine the Minimum Lethal Dose for Mice.

Weight of Mouse in Grams	Dose cc./Gm.	Results After One Hour Observation
26.0	0.00300	Death
18.0	0.00300	Death
25.5	0.00235	Death
16.5	0.00235	Death
18.5	0.00230	Death
22.0	0.00230	Death
26.5	0.00230	Recovery
17.5	0.00230	Death
21.5	0.00230	Death
19.0	0.00230	Death
20.0	0.00230	Death
20.5	0.00230	Death
19.5	0.00230	Death
25.0	0.00230	Death
22.0	0.00230	Death
19.0	0.00230	Death
24.0	0.00225	Recovery
28.5	0.00225	Death
20.0	0.00225	Death
21.0	0.00225	Recovery
19.5	0.00225	Death
22.0	0.00225	Death
27.5	0.00225	Recovery
27.0	0.00225	Death
19.0	0.00225	Death
16.5	0.00225	Death
21.0	0.00225	Recovery
20.5	0.00225	Recovery
17.5	0.00220	Recovery
20.5	0.00220	Recovery
15.5	0.00220	Death
26.0	0.00220	Death
16.0	0.00200	Recovery
25.5	0.00200	Recovery
20.0	0.00200	Death
24.5	0.00170	Recovery
24.0	0.00170	Recovery
24.5	0.00150	Recovery
22.5	0.00150	Recovery
26.5	0.00150	Recovery

TABLE 26 b

Summary of Table 26 a. The Minimum Lethal Dose for Preparation 114.

Dose cc./Gm.	Number of Injections	Results of Injections	
		Death	Recovery
0.00300	2	2	
0.00235	2	2	
0.00230*	12	11	1
0.00225	12	7	5
0.00220	4	2	2
0.00200	3	1	2
0.00170	2		2
0.00150	3		3

* Minimum Lethal Dose

TABLE 27

A Comparison of the M.E.M.D. for Pigeons and the M.L.D. for Mice.
Preparation 104 is used as 100%.

Preparation	M.E.M.D.	Comparative Potency	M.L.D.	Comparative Potency
102	0.45	189%	0.00075	203%
104	0.85	100%	0.00155	100%
106	0.35	242%	0.00080	259%
107	0.95	90%	0.00230	68%
108	0.90	106%	0.00165	94%
110	0.65	130%	0.00165	94%
114	0.65	100%	0.00230	68%

INVESTIGATION OF THE ALKALOIDS

EXTRACTION AND ISOLATION

The drug used for this part of the work was from a 100 pound lot obtained from a firm that supplies crude drugs. It was in the form of a number 20 powder and had a moisture content by the toluene method of 7.5 per cent. The total ash was 6.4 per cent and the acid insoluble ash was 4.8 per cent. This acid insoluble ash content is quite high in view of the fact that the N.F. limit is 2 per cent. A tincture made from a representative sample has a N.E.M.D. of 0.85.

The procedure given by Sayre and Watson (10) was followed in isolating those alkaloids reported by them. A 10 Kg. sample of the drug was used and this was extracted by fractional percolation with 70 per cent alcohol.

A few remarks regarding the extraction may be of interest. For moistening, 700 cc. per Kg. were used. The first portion of 5 Kg. was packed into four percolators and the drug extracted until the 2 L. reserve and the 5 portions of 3 L. each were obtained. Tests with Mayer's Reagent on the last part of percolate did not give a precipitate. About five liters remained in the percolators and this was drawn off and used as menstruum on the second portion of drug (packed in 3 percolators), after the 5 portions of 3 L. each had been used. Although the reserve of 3 L. and the 5 portions of 2 L. each had been gathered the extract still gave a precipitate with Mayer's Reagent, and it was necessary to add about seven liters of menstruum before extraction was accomplished. Extraction was called complete when Mayer's Reagent gave only a faint

turbidity. All the extract was added in order to the third portion of drug (packed in 2 percolators) and it was necessary to add 6 L. of fresh menstruum before complete extraction was obtained. Thus it may be seen that it was necessary to add considerably more menstruum than is called for in the N.F. specifications for this method of extraction. No attempt was made to assay the different portions so no conclusions can be drawn regarding the per cent of alkaloids extracted before addition of the extra menstruum. However, it seems that the fractional percolation method using 70 per cent alcohol does not give complete extraction with this lot of Gelsemium, but more study would be necessary to make any definite statements.

Since the procedure for isolation as given by Sayre and Watson (10) was followed exactly it is not necessary to repeat the details here. There was no difficulty in following the steps given by these authors, but there was considerable difficulty with emulsions in the chloroform extractions which was finally overcome by gentle shaking, long waiting, and increasing the solvent.

GELSEMIC ACID (scopoletin, $C_{10}H_8O_4$) was removed and purified by crystallization from alcohol. The uncorrected melting point was found to be $203^{\circ}C$. which seems to be close enough to the reported (11) melting point of $204^{\circ}C$. to justify considering them as the same, since the material met other descriptions such as fluorescence in alcoholic solutions, green color with ferric chloride, and needle crystals. No attempt at chemical identification was attempted since that is beyond the scope of this problem and is not necessary for the purpose. About one gram of the purified material was obtained.

GEISEMINE was isolated in the form of the hydrochloride. Its uncorrected melting point of $318^{\circ} - 320^{\circ} \text{ C.}$ is in conformity with the reported melting point of about 300° C. The melting point of a sample of Merck's Gelseminine HCl. (Gelsemine HCl) was also determined and found to be $317^{\circ} - 318^{\circ} \text{ C.}$ The exact melting point is rather difficult to determine since the material begins to darken well below the melting point and continues darkening until it is quite black at the melting point. Sayre and Watson reported 8 Gm. of this alkaloidal hydrochloride and 7.5 Gm. were obtained in this work. Its reported empirical formula is $(\text{C}_{20}\text{H}_{22}\text{O}_2\text{N}_2)$.

SEMPERVIRINE was isolated in the form of the nitrate which crystallized from alcohol in yellow needle-shaped crystals. The uncorrected melting point was $278^{\circ} - 280^{\circ} \text{ C.}$ with decomposition, which seems to be in agreement with the reported (12) melting point of $280^{\circ} - 282^{\circ} \text{ C.}$ with decomposition. The material begins to darken at about 250° C. and there is continued darkening until it is black at the melting point. This alkaloid is fluorescent, a fact which was not mentioned by Sayre and Watson but was spoken of by Hasenfratz (13) who worked out the empirical formula $(\text{C}_{19}\text{H}_{16}\text{N}_2)$.

GEISEMIDINE AND GEISEMOIDINE, the two remaining alkaloids reported by Sayre and Watson were obtained in the form of the hydrochlorides. Since there are no melting points available these could not be checked but their forms, colors, solubilities, reactions, etc. were the same as those published so for the purposes of this investigation they were accepted as being the same alkaloids as reported by Sayre and Watson.

GEISEMICINE $(\text{C}_{20}\text{H}_{25}\text{O}_4\text{N}_2)$, a third crystalline alkaloid from Gel-

seminum has been reported by Chou (14) who gave in this and a subsequent paper (15) his procedure for separating the alkaloids as found by him. He agreed with Sayre and Watson on the identity of gelsemine, reported gelsemicine as the new alkaloid and further reported that he did not feel that his sempervine was the same as the previously reported sempervirine although the appearances and melting points of the two were in close agreement. Hasenfratz (15) who published the formula for sempervirine said that they seemed to be the same, and later writers appear to consider them as identical. Chou also reported an amorphous alkaloid for which he gave no name.

In order that all the alkaloids which have been reported might be studied, an attempt was made to follow the procedure outlined by Chou. He used 48 Kg. of drug but only 10 Kg. were used in this investigation. The menstruum was 95 per cent alcohol and it required about 52 L. of this to get an extract which did not give a precipitate with Mayer's Reagent.

It has been difficult to follow the procedure outlined by Chou because of the incompleteness in description of some steps and thus far no gelsemicine has been obtained. Chou did not state in his report how much of the alkaloid was obtained from the 48 Kg. of drug, but the quantity was probably small and this smallness in quantity may account, in part, for the inability to obtain it from the 10 Kg. of drug. However, this does not seem to be the entire explanation since other alkaloids were not obtained in quantities comparable to those from the procedure of Sayre and Watson. About 7.5 Gm. of gelsemine HCl. were obtained from their procedure while less than one gram of gelsemine could

be obtained in pure form by Chou's procedure. Also only a few milligrams of impure sempervine nitrate (melting point 276° C.) was obtained and none of the amorphous alkaloid was collected after following as near as possible the procedure he described. The material obtained did not give positive tests with alkaloidal reagents.

TESTS FOR EMETIC ACTION

These tests were made by injecting pigeons with solutions of the isolated principles in the same manner as was used in the injection with tincture. The object was to determine what principle or principles caused the emetic action. The strengths of the solutions used is given under each heading to follow. The solutions were made either on the day of injection or on the day before.

GELSEMIC ACID. Solutions varying in strength up to 20 mg. per cc. were made with this principle, using 50 per cent by volume alcohol as the solvent. The crystals were dissolved in the alcohol and then the water was added to bring the solution to volume. Doses were administered in increasing size until a dose of 20 mg. per Kg. had been injected into 8 pigeons without any emetic action. Since this quantity is several times the quantity that could be expected to be in an emetic dose of the tincture, it is felt that this principle can not be responsible for any of the emetic action.

GELSEMINE HCL. The solutions were made using water as the solvent. The starting dose was 0.1 mg. per Kg. and the size of the dose was increased until 8 pigeons had received 20 mg. per Kg. without giving any emesis. The total alkaloids contained in the tinctures is somewhere in

the neighborhood of 0.05 Gm. per 100 cc. which would be 0.5 mg. total alkaloids per cc. of tincture. Since in no case has a tincture been found with a M.E.m.D. of as much as 1 cc., it may be assumed that 0.5 mg. per Kg. of total alkaloids would produce emesis in all cases. Thus since a dose of gelsemine HCl of 20 mg. per Kg., which is 40 times the amount of total alkaloids assumed to be able to cause emesis, did not cause emesis, it seems reasonable to assume that this alkaloid is not responsible to any appreciable extent for the emetic action of the drug.

SEMPERVIRINE NO_3 . A solution of this was made so that each cc. contained 1.6 mg. in 50 per cent alcohol. The salt was dissolved in hot alcohol and warm water was added to bring the solution to volume. Doses ranged from 0.8 mg. to 2.8 mg. per Kg. with results as shown in the following table:

TABLE 28
Results of Injections of Sempervirine NO_3

Dose mg./Kg.	Number of Injections	Emesis	No Emesis
0.8	1		1
1.6	3		3
2.4	4	1	3
2.6	2		2
2.7	12	3	9
2.8	12	10	2

From this it may be seen that sempervirine NO_3 has a M.E.m.D. of 2.8 mg. The emetic action is prompt, occurring in from 5 to 7 minutes. Although the size of this dose is about five times larger than the size

of the dose of total alkaloids considered as sufficient to give emesis, it can be seen that this alkaloid is partially responsible for the emetic action. Since the alkaloid does not likely occur in the drug in the form of the nitrate no direct quantitative comparison can be made. The material obtained by following Chou's procedure had a melting point a little under the reported one so the salt is doubtless impure, but a few injections were made using it. The dose of 2.8 mg. per Kg. did not give emesis in four injections but nausea was noticeable. However, the number of injections is too small to be of value, but since the quantity was not sufficient to make further injections no further data could be obtained. It seems reasonable to consider the two as the same alkaloid since Hasenfratz (13) reported that he believed them to be identical and since subsequent workers consider the same (11).

GELSEMAIDINE HCL. The solution of this was made with water since it is easily soluble in this, in fact it is hygroscopic. Doses ranging from 0.5 mg. per Kg. to 20 mg. per Kg. were administered without obtaining any emetic action. Since this is several times the quantity that could be expected to be found in an emetic dose of the tincture, it is not believed that this alkaloid is responsible to any appreciable extent for the emetic action.

GELSEMAIDINE HCL. The solutions used contained 0.6 mg. in each cc. and had water as the solvent. The doses ranged from 0.6 mg. per Kg. to 1.2 mg. per Kg. with the results as shown in the following table:

TABLE 29
Results of Injections of Gelsemoidine HCl

Dose mg./Kg.	Number of Injections	Emesis	No Emesis
0.6	3	1	2
0.75	3	1	2
0.90	12	8	4
0.95	12	5	7
1.14	4	2	2
1.20	8	7	1

From this it is seen that the amorphous alkaloid of Sayre and Watson has a M.E.M.D. of approximately one mg. per Kg. The emetic action was not quite as prompt as that for sempervirine NO₃ since this required from 10 to 15 minutes. The amorphous alkaloid called Gelsemoidine by Sayre and Watson is partially responsible for the emetic action of the drug.

GELSEMICINE. Since none of this alkaloid has thus far been isolated in this investigation, it is not possible to give any tests for emetic action. However, it may be said that it has been reported (16) that gelsemicine produces emesis in pigeons, but up to this time it is not possible to say what the emetic dose is.

DISCUSSION OF THE EMETIC PRINCIPLES

Very little experimental work has been published which gives the action of the various alkaloids, but it is generally considered that gelsemine is not active as far as mammals are concerned, although Chen (14) has shown that 7 mg. into one cat gave a fall in blood pressure and

Hamet (3) states that it is active in guinea pigs but does not say in what way. It does produce mydriasis locally. It seems from the literature that this must not be responsible to a very great extent for the therapeutic action of the drug. In the present study it has been found that in doses of 20 mg. per Kg. this is not responsible for the emetic action.

Sempervirine is reported as active by Hamet (2) and Hou (17). Since this alkaloid does produce emesis in pigeons it seems that this method would serve to measure its activity.

Gelsemidine does not produce emesis in pigeons in doses of 20 mg. per Kg. Its action has not been published except on frogs (10), but it does not seem that this is responsible to any extent for therapeutic action.

The only physiological action published for the amorphous alkaloid, Gelsemidine, is its quieting action on frogs. The drug is used for its quieting action. Since it has not been determined just what this substance is, nothing definite can be said about it for it may contain some of another principle. It does produce emesis in pigeons so that this method seems to measure this alkaloid.

Gelsemidine is the most toxic alkaloid in the drug and has effects on respiration (16). Since this produces emesis in pigeons it would seem that the method would measure its activity.

The quantities of the two alkaloids which were found to produce emesis in this investigation is larger than could be responsible for the emesis of the drug, but Gelsemidine has been said to produce emesis also although the dose is not known. Since the composition of the amorphous

alkaloid is not known this may not be a pure substance. There is also the possibility of a synergistic action of those alkaloids which do provoke the emesis.

The pigeon emesis method does measure activity of those alkaloids which have been demonstrated to give physiological activity. In those alkaloids which do not give emesis the physiological activity has not been sufficiently substantiated. Thus it seems reasonable to assume that emesis in pigeons is a result of the action of those alkaloids which give the therapeutic action of the drug and that the method is suitable for assay.

CONCLUSIONS

From the further data obtained in this investigation it has been shown that with Gelsemium pigeons do not become more sensitive to repeated injections than the limits of experimental error.

The method has an accuracy of from 5 to 20 per cent.

Four preparations made from drug grown in Florida seem to indicate that such drug is more potent than that ordinarily found on the market.

Frogs are not a suitable animal for the assay of Gelsemium.

The 75 per cent M.L.D. for mice and the M.E.M.D. for pigeons run somewhat parallel, indicating that pigeon emesis does measure activity of the drug.

Pigeon emesis measures the activity of those alkaloids which have been shown sufficiently to have physiological action for mammals.

Pigeon emesis serves to measure more adequately than any other method so far suggested the activity of Gelsemium. The method possesses the following desirable qualities: economy, simplicity, rapidity and a definite end point.

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BIOGRAPHY

Lea Gene Gramling was born at High Springs, Florida, November 3, 1908. He received his high school education at Plant City High School, Plant City, Florida and entered the College of Engineering of the University of Florida in the fall of 1927 but transferred to the College of Pharmacy the following fall. He received the Certificate of Graduate in Pharmacy in June 1931. In June 1935 he received the degree of Bachelor of Science in Pharmacy with High Honors and in 1936 he received the degree of Master of Science in Pharmacy. He has held Graduate Scholarships in Pharmacology for the school years of 1935 - 36, 1936 - 37 and 1937 - 38.

He has been a registered pharmacist, by examination, in the State of Florida since 1931 and has been employed in several drug stores in the state as pharmacist, assistant manager or as manager.

He holds a commission as Second Lieutenant in the Medical Administrative Reserves. In 1935 he was the recipient of the D.W. Ramsaur Scholarship Medal. He is a member of Delta Chi social fraternity and of the following: Phi Kappa Phi, Rho Chi, Phi Sigma, Phi Eta Sigma, Gamma Sigma Epsilon, and Seaboard and Blade.

This dissertation was prepared under the direction of the Chairman of the candidate's Supervisory Committee, and has been approved by all members of the Committee. It was submitted to the Graduate Council and was approved as partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Date August 27, 1938.

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